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Seasonality of Coliform Bacteria Detection Rates in New Jersey Domestic Wells

by Thomas B. Atherholt¹, Nicholas A. Procopio², and Sandra M. Goodrow¹

Abstract

It is important that indicators of fecal pollution are reliable. Coliform bacteria are a commonly used indicator of fecal pollution. As other investigators have reported elsewhere, we observed a seasonal pattern of coliform bacteria detections in domestic wells in New Jersey. Examination of a statewide database of 10 years of water quality data from 93,447 samples, from 78,207 wells, generated during real estate transactions, revealed that coliform bacteria were detected in a higher proportion of wells during warm weather months. Further examination of the seasonal pattern of other data, including well water pH, precipitation, ground and surface water temperatures, surface water coliform bacteria concentrations, and vegetation, resulted in the hypothesis that these bacteria may be derived from nonfecal (or environmentally adapted) as well as fecal sources. We provide evidence that the coliform seasonality may be the result of seasonal changes in groundwater extraction volumes (and to a lesser extent precipitation), and temperature-driven changes in the concentration of surface or near-surface coliform sources. Nonfecal coliform sources may not indicate the presence of fecal wastes and hence the potential presence of pathogens, or do so in an inconsistent fashion. Additional research is needed to identify the sources of the coliforms detected in groundwater.

Introduction

Contamination of groundwater (GW) by fecal wastes is a health risk due to the occurrence of pathogenic microorganisms that are often present in these wastes (Raina et al. 1999; Said et al. 2003; Borchardt et al. 2012). Such contamination is usually determined by testing water for the presence of “indicator” bacteria such as total coliform (TC), fecal coliform (FC), or *Escherichia coli* (*E. coli*) bacteria (Leclerc et al. 2001; Sadowsky and Whitman 2011). Coliform bacteria are always present in untreated wastes from humans and warm-blooded animals and therefore these bacteria are used to indicate the potential presence of pathogens. The U.S. Environmental Protection Agency (USEPA) recommends the private well owners to test their wells for TC bacteria, nitrates, total

dissolved solids, and pH at least once a year, and more often under certain circumstances (USEPA 2002, 2012).

However, indigenous or environmentally adapted coliform bacteria, including *E. coli*, can also be found in nonfecal sources such as soils, sands, sediments, and vegetation (Byappanahalli and Ishii 2011; Luo et al. 2011). Therefore, when coliform bacteria are detected in a domestic well, their source is uncertain.

The efficiency with which coliform bacteria can be transported from surface and subsurface sources to GW, and hence increase in their concentration in domestic wells, is determined by a number of factors including the amount of precipitation (Hynds et al. 2012), the geological formation in which the well is located (Embrey and Runkle 2006; DeSimone 2009; Toccalino et al. 2010; Atherholt et al. 2013), well depth (Glanville et al. 1997; Gonzales 2008; Allevi et al. 2013), and deficiencies in well construction or integrity (Bacci and Chapman 2011; Swistock et al. 2013; Hynds et al. 2014). Once in the GW, the efficiency with which these bacteria are detected is influenced by the laboratory and type of detection method used (Francy et al. 2000; Griffith et al. 2006; Atherholt et al. 2013), and also by the number of times a well is sampled (Atherholt et al. 2015).

A seasonal difference (summer peak) in rates of acute gastrointestinal illness has been observed among populations who drink water from public and private GW sources (Galway et al. 2014; Wallender et al. 2014). These authors suggested temperature and/or precipitation and

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runoff as possible influencing factors, although seasonal influence of foodborne sources or other factors (see Addiss et al. 1992; Post et al. 2011) could not be ruled out.

Richardson et al. (2009) conducted a large survey of ground and surface waters in England and observed a warm weather increase in coliform detection rates in GW. Earlier reports from England, Scotland, and Canada also observed a similar seasonal pattern (Raina et al. 1999; Rutter et al. 2000; Reid et al. 2003). Richardson et al. (2009) speculated that the mechanism(s) underlying the seasonal trend may have involved seasonal agricultural practices and/or climate factors.

We also observed a similar seasonal difference in coliform detection rates in New Jersey (NJ) domestic wells in this study. However, since 97% of the wells examined in this study are located in non-agricultural areas (88% on residential land), seasonal agricultural practices would be expected to have little, if any, effect on the seasonal difference in coliform detection rates in GW. Therefore, we hypothesized that climate or other factors are responsible for the seasonal difference in GW coliform detection rates in NJ.

The NJ Private Well Testing Act (PWTA) went into effect in 2002 (New Jersey Department of Environmental Protection [NJDEP] 2002). The Act specifies that buyers or sellers in real estate transactions, and landlords of properties with a well not required to be tested under other state law, must test and share information regarding the quality of the well's source water. Untreated water must be tested by a state-certified laboratory for 30 or 31 (depending on county location) health-related chemical and microbiological parameters (coliform bacteria), as well as for pH, iron, and manganese (Atherholt et al. 2009). If TC bacteria are detected, that sample is further tested for the presence of either FC or *E. coli* (FC/EC) bacteria. FC bacteria are a more fecal-specific subset of the TC group of bacteria (Leclerc et al. 2001). *E. coli* bacteria comprise the majority, often 75% or more, of the FC group of bacteria in most but not all cases (Francy et al. 1993; Cude 2005). PWTA data are provided to the buyer and seller of the property and are also submitted electronically to the NJDEP.

Two previous studies used the PWTA coliform data to examine the influence of several factors on GW coliform detection rates. These factors included the geologic setting of a well, the laboratory and method used to generate the data (Atherholt et al. 2013), and the number of times a well is sampled (Atherholt et al. 2015).

This study examined the role of climate and the seasonal difference in GW extraction volumes in determining the warm-weather increase in coliform detections in NJ domestic wells. The increase in coliform detections in GW in warm weather is a reflection of a seasonal increase in coliform concentrations. Depending on the sources of the detected coliforms, the seasonal change in coliform concentrations may or may not correspond to a similar change in waterborne pathogen concentrations. We review the potential coliform sources and explain why some may

not indicate the potential presence of pathogens or do so in an inconsistent fashion. We also verified that the observed seasonality was not a sampling artifact of previously examined influences on coliform detection rates in GW, such as the location of a well in certain types of geology (Atherholt et al. 2013), the number of times a well is sampled (Atherholt et al. 2015), or a seasonal difference in the number of sampled wells that were located in agriculture land.

Study Area

The northern half of NJ is composed of sedimentary, igneous, and metamorphic bedrock (BK) in three geologic provinces: Piedmont, Highlands, and Valley & Ridge (Figure 1). Aquifers in the BK occur within strata that can be broadly described as fractured BK that are covered, in some areas, with unconsolidated regolith or glacial materials (Serfes 1994, 2004). The southern half of NJ is part of the North Atlantic Coastal Plain (CP). Aquifers in the CP are contained within thick sequences of sands, silts, and clays that slope downward to the south and east and in increasing thickness toward the Atlantic Ocean (Sugarman et al. 2005). The boundary where the CP meets the BK is known as the "Fall Line."

Data

Well Sampling and Testing

From October 1, 2002 through September 20, 2012, untreated water from 78,207 domestic wells (93,447 samples including wells that had been sampled two or more times) was analyzed for TC bacteria by one of 39 laboratories using one of the methods (Clesceri et al. 1998) shown in Table 1.

The number of wells and samples analyzed during each month of the year are shown in Table 2.

All samples detected with TC bacteria were further analyzed for either FC or *E. coli* (FC/EC) bacteria, as permitted by the PWTA and depending on individual laboratory method certifications. All methods are approved by the USEPA and the NJDEP for use in drinking water testing, and all have a detection limit of one viable organism (reported as CFU or MPN in quantitative assays) per 100 mL. Although some coliform data were quantitative, most laboratories reported presence/absence data only, as permitted by the PWTA. The quantitative data were not analyzed as such in this study but were included as either present or absent from the sample.

If wells had treatment in place (e.g., disinfection, water softeners), the water was sampled prior to treatment. All sampling and analytical laboratory personnel were trained in proper sampling protocols, and all laboratories are certified by the NJDEP for the method(s) used. Certification includes routine quality control (e.g., replicate testing, media qualifications, sterility controls, and positive and negative control samples, including matrix

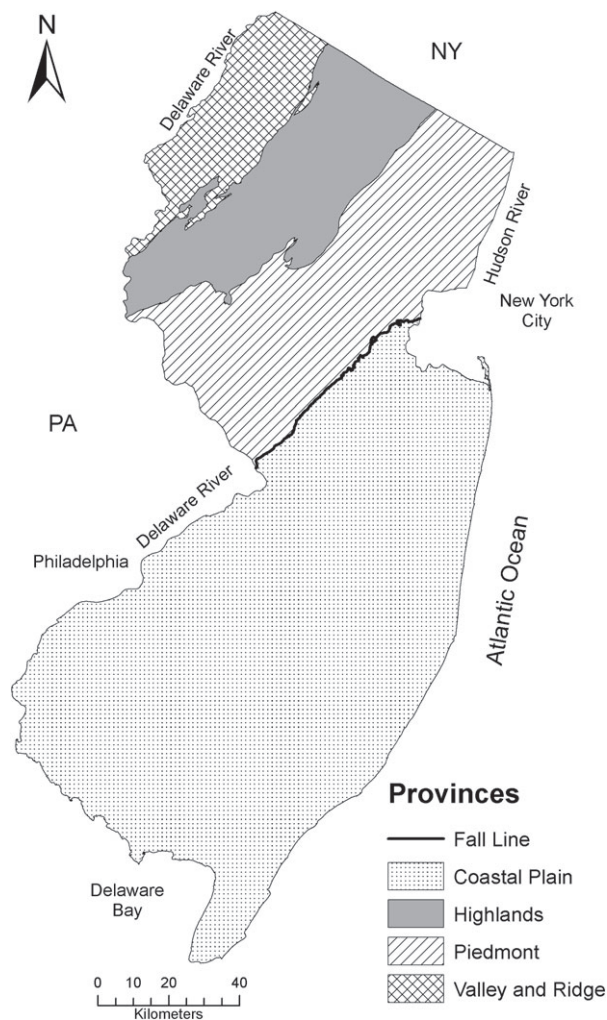


Figure 1. Geologic regions of NJ. The Highlands, Piedmont, and Valley and Ridge provinces comprise the BK region. The CP province occupies the entire area of NJ south of the Fall Line.

spike samples) and periodic proficiency testing evaluations for each method. Samples were held for 6 h or below and between 4 and 10°C following the collection and before the start of sample analysis.

In this analysis, we define detection rate as the number of wells in which coliform bacteria were detected divided by the number of wells sampled. Because coliform detection rates in the CP and BK regions are so different (Atherholt et al. 2013, 2015), data are presented separately for the two regions (Table 1).

The PWTa electronic data are not subject to quality control other than well location and test method verification, and hence some of the data are likely suspect. However, the database is very large, and, since the data were submitted by many different laboratories (99.9% of the data was submitted by 30 laboratories that each analyzed 100 or more samples), submission of some inaccurate data by one or a few laboratories should have minimal impact on overall database accuracy. The limitations and strengths of the data have been discussed in detail previously (Atherholt et al. 2013, 2015).

pH

In addition to coliform bacteria data, the PWTa database includes pH data, generated at the time of sampling. Within each geologic region, the wells were divided into two groups: those with a pH below 7.0 and those with a pH of 7.0 or higher. Data from 413 of the 93,447 samples (0.4%) could not be used either because pH data were not provided ($n = 228$; some samples were tested for coliform bacteria only because the expiration period for valid coliform test data is shorter than that of the other PWTa parameters), because of unrealistic pH values below 3 or above 10 ($n = 102$), or because the data were unusable due to the reporting of “less than” values ($n = 83$).

Precipitation

Twenty-four-hour total precipitation values from September 2002 through December 2012 were collected from 66 monitoring stations located in NJ and also from the nearby Philadelphia International Airport in PA. The data were obtained from the National Weather Service (NWS: www.ncdc.noaa.gov; Table S1 and Figure S1, Supporting Information). Some of the data had been “flagged,” but not excluded, by the NWS as failing spatial or temporal consistency checks. Following our own temporal and spatial consistency check, we excluded some but not all of the NWS flagged data along with some other data that failed these same quality control checks. In all, 151 of the 181,149 (0.08%) precipitation values were excluded. No data were excluded from 40% of the sites, $\leq 0.1\%$ of the data were excluded from 37% of the sites, and 0.2 to 1.4% of the data were excluded from the remaining 22% of the sites.

Long-term NJ average monthly precipitation totals from 1895 to 2015 were obtained from the NJ State Climatologist website (New Jersey State Climatologist 2015).

Well Distance from Surface Waterbodies

The distance of each well from a reservoir, lake or pond, canal, or stream or river was determined using NJDEP’s “Stream Network 2002” and “Waterbody 2002” data from the NJDEP website (NJDEP Bureau of Geographic Information System 2015) using the buffer tool in ArcView 10.2 Geographic Information System (GIS) software (ESRI, Inc., Redlands, California). Each well was categorized according to its distance from the nearest waterbody as follows; 1 to 100, 100.1 to 300, 300.1 to 500, 500.1 to 1000, or greater than 1000 feet (0.3 to 30.5, 30.51 to 91, 91.1 to 152, 152.1 to 305, or greater than 305 m).

Surface Water Temperatures

Monthly mean surface water temperature data from 14 monitoring locations in the BK region (from 11 different streams) and 9 locations in CP region (from 8 different streams) for the years 1968 to 2013 were downloaded from the U. S. Geological Survey (USGS) National Water Information System database. Because the

Table 1
Coliform Bacteria Test Methods Used in the NJ Private Well Testing Act^{1,2}

Method	NJ			BK Region			CP		
	Total Number of Laboratories	Total Number of Samples	Number of Laboratories Testing 100 or More Samples	Total Number of Laboratories	Total Number of Samples	Number of Laboratories Testing 100 or More Samples	Total Number of Laboratories	Total Number of Samples	Number of Laboratories Testing 100 or More Samples
TC method									
Fermentation ("Ferm"; SM9221B or SM9221D)	5	5201	4	4	3721	3	3	1480	2
Membrane filtration ("MF"; SM9222B; M-Endo agar)	20	33,142	13	17	5931	6	17	27,211	9
Enzyme substrate ("ES") ³	26	55,107	17	24	36,206	15	19	18,898	8
FC method									
Fermentation (multiple- or single-tube, SM9221E) ⁴	15	28,874	13	11	3322	5	15	25,552	9
EC method									
Fermentation (SM9221F) ⁴	6	9460	3	6	6321	3	4	3139	2
Enzyme substrate ("ES") ³	26	55,104	17	24	36,206	15	19	18,898	8

¹Clesceri et al. 1998.

²Data from 39 laboratories are included. Several laboratories used more than one method.

³SM9223B (Colilert®, Colilert-18®, or Colisure®; IDEXX Corp., Westbrook, ME), m-ColiBlue24® (Hach Co., Ames, IA), or Colitag® (CPI International, Santa Rosa, CA).

⁴FC/EC method unknown for four laboratories (eight samples). TC test result was negative.

Table 2
Number of Samples and Wells Analyzed Each Month

Month	January	February	March	April	May	June	July	August	September	October	November	December	Total
Samples ¹	6008	6365	8475	8542	9309	9380	8603	8564	7254	8171	7036	5740	93,447
Wells ²	5051	5337	7050	7176	7738	7838	7116	7041	6074	6990	5940	4856	78,207

¹Includes data from wells sampled two or more times.

²Repeat sample data excluded.

data from each station were from a different monitoring period (different years), median values were calculated from the monthly means of each site for each of the two regions.

These data are provided as a proxy for what we assume are similar temperatures in other surface and near-surface potential coliform sources located in each region, including lakes, surface and near-surface soils and rock strata, and well casing and grout biofilms.

Ground Water Temperatures

Routine-sample GW temperature data generated by the USGS-New Jersey Water Science Center were downloaded from the National Water Quality Portal website (www.waterqualitydata.us/). Data from 14,435 samples from 4250 wells (1 to 80 samples per well; 75% of wells sampled three times or less) collected between 1948 and 2015, were analyzed. Because GW temperature increases with depth below approximately 500 feet (152 m) (a few wells with depths >3000 feet [914 m] had temperatures over 40 °C), and because the deepest noncommunity (e.g., schools, businesses) public well in NJ is 1205 feet (367 m) (well depth information is not included in the PWTA database), wells with a depth greater than 1000 feet (305 m) were excluded from this analysis.

Surface Water Coliform Bacteria

Surface water TC and FC data from NJ streams, generated by the USGS, were downloaded from the Water Quality Portal website (www.waterqualitydata.us/). TC data from 1968 to 1975 and FC data from 1972 to 1996 were analyzed. Summary statistics were generated for each month's data. The FC data included multiple left- and right-censored values. More recent data were available only for the summer bathing season, and therefore were not utilized.

Vegetation Index

Monthly changes in the amount of vegetation cover in NJ were determined using National Aeronautics and Space Administration's (NASA) Moderate Resolution Imaging Spectroradiometer Terra satellite data (NASA 2016). The 0.05° (latitude/longitude; 5600 m) climate modeling grid (CMG) resolution, enhanced vegetation index (EVI), monthly composite product ("MOD13C2") was used.

Vegetation index products are cloud-free measurements of canopy "greenness," a composite property of leaf area, chlorophyll and canopy structure, based on

atmosphere- and background-corrected bidirectional surface reflectance of red (648 nm), near infrared (848 nm), and blue (470 nm) wavelengths. The global index (unitless) scale ranges from −2000 to +10,000. Values close to zero imply no vegetation, dead vegetation, only soil, turbid water, etc. Negative numbers imply the presence of snow or ice, or (less likely) thick white clouds or dark blue water. The NASA (2016) website provides additional details of these products.

For the period from October 2002 through September 2012, the 855 CMGs ("grids") of the EVI layer covering the state of NJ were extracted from the global files using the ArcView GIS software. The 855 index values for each month were imported into Microsoft Excel files and the monthly values for all 10 years were composited on a per-month basis ($n = 8550$ for each month).

Land Use/Land Cover

Land use/land cover (LULC) category data from 2002, 2007, and 2012 were obtained from the NJDEP website (NJDEP Bureau of Geographic Information System 2015). The LULC category (agriculture, barren, urban, forest, water, or wetland) that encompassed the location of each well was identified using the ArcView GIS software. Wells located in the residential subcategories of the urban LULC category were further identified. Water and wetlands were combined into one category but were not included in analyses because of the low number of wells located in this category and the likelihood, based on interpretation of aerial photographs from these years, that many of the wells placed in this class are an artifact of LULC mapping errors. The LULC category for each well was based on data from the relevant time periods that are as follows: wells sampled between October 1, 2002 and March 31, 2005 – 2002 LULC data; wells sampled between April 1, 2005 and March 31, 2009 – 2007 LULC data; and wells sampled on or after April 1, 2009 – 2012 LULC data.

Statistics

Descriptive statistics for uncensored data were generated using Statistica 12.0 (StatSoft Inc., Tulsa, Oklahoma), including monthly 25th, 50th, and 75th percentile values of the surface water coliform data. Because the FC data contained multiple left- and right-censored (< and >) data to varying extents, percentile values were calculated using the Kaplan-Meier Turnbull procedure (Helsel 2012) within the Survival package (v2.38.3) of the R

statistics software (v3.1.2). Comparisons of proportions were evaluated using Chi-square tests of independence using R. Post hoc assessments of significant Chi-square tests were performed to identify the pairwise differences among test groups. Post hoc tests utilized the sequential Bonferroni (Abdi 2010) or False Discovery Rate (Benjamini and Hochberg 1995) methods and were computed using the Fifer package (v1.0) in R.

Graphs were made using SigmaPlot 12.0 (SysStat Software, Inc., San Jose, California) and maps were made using ArcView 10.2.

Results

Seasonality of PWTa Coliform Rates

As shown in Figure 2, the percentage of wells in which TC or FC/EC bacteria were detected showed a seasonal pattern during each year of the study's 10-year period, with the greatest percentage of coliform-positive wells occurring during the warm weather months. The data in Figure 2 and the hypothesis that both TC and FC/EC are dependent on a seasonal basis are statistically supported (Chi-square = 1091, $df = 3$, $p < 0.0001$ for TC and Chi-square = 366.1, $df = 3$, $p < 0.0001$ for FC/EC). Post hoc tests revealed significantly higher rates of TC and FC/EC in the warm seasons of summer and fall (June through November) than in either winter or spring (December through May).

The minimum, mean, and maximum number of wells sampled per month were 106, 379, and 849 in the BK region and 55, 396, and 825 in the CP. The large difference in detection rates between the BK and CP regions is due to the large difference in the vulnerability of the consolidated strata in the BK region (igneous, metamorphic, and sedimentary rock, including areas with little or no overburden due to past glacial activity), compared to that of the unconsolidated layers of silt, sand, and clay that comprise the CP (Atherholt et al. 2013).

Individual Laboratory and Method Seasonality Data

The differences in percentage of coliform detections for each month could be affected by differing proportions of data derived from each laboratory because locations of well populations analyzed and methods used (with differing overall detection frequencies) vary among laboratories. Therefore, it was important to determine whether this seasonal pattern occurred on an individual laboratory/method basis. The monthly percentage of coliform-positive wells in both the BK and CP regions was analyzed separately for each laboratory that analyzed 100 or more samples per month. The TC bacteria results are shown in Figure 3. The data show that although the percentage of positive wells observed each month differs among the laboratories and methods used, a seasonal pattern was present in results from almost all laboratories.

The TC detection rate from Lab BB, which predominantly used the MF method, was significantly below those of the other laboratories testing in the same areas of the

BK and CP regions. (Note: This laboratory used the MF method from October 2002 to July 2010. The laboratory began using an ES method in February 2004, used it sparingly until July 2010, and then exclusively from September 2010 through September 2012.) In the BK region, Lab BB's yearly TC detections rates using the MF method were: 2002 to 2005, 2.7%; 2006 to 2008, 8.0%, 2009 to 2010, 16.1%. Thus, based on comparison of these yearly detection rates to yearly rates from the other laboratories, Lab BB's electronically submitted data between 2002 and 2008 are suspect. If Lab BB's data are removed from the database, then the previously reported (lower) TC bacteria detection rates using the MF method (Atherholt et al. 2013) are comparable to the ES method detection rates.

pH

The monthly average pH of well water in the BK and CP region is shown in Figure 4. In the BK region, a lower proportion of the sampled wells were basic ($pH \geq 7.0$) during warm weather than in cold weather. The monthly difference was statistically significant (Chi-square = 55.67, $df = 11$, $p < 0.0001$). This observation was primarily driven by conditions in April, July, and August. No such seasonal effect was observed in the CP.

Precipitation

The monthly mean precipitation amounts over the 10-year monitoring period of this study, as well as monthly mean amounts over 118 years in NJ, are shown in Figure 5. A seasonal precipitation pattern with increased monthly means in the summer and autumn is present in both the record covering this study period and the past century.

Well Distance from Surface Waterbodies

The percentage of wells in which TC bacteria were detected, in wells located within the indicated distance from the nearest waterbody type, is shown in Figure 6. Comparisons using Chi-squared tests of independence suggest that coliform bacteria are detected more frequently in wells that are more proximate to lakes, ponds, or reservoirs in the BK region.

Surface and Ground Water Temperature

Monthly median surface and ground water temperatures in NJ are shown in Figure 7. In both regions, shallow wells (1 to 50 feet [0.3 to 15 m]) were 1 to 3 °C colder than deeper wells (>50 to 1000 feet [15 to 305 m]) in cold weather, and 1 to 2 °C warmer in warm weather (data not shown). Monthly GW temperature does not vary greatly but a slight increase is evident in the warmer months.

Surface Water Coliform Bacteria

Monthly river and stream coliform concentrations (USGS) and GW detection rates (PWTa) are shown in Figure 8. The USGS coliform sampling locations are shown in Figure S2. A clear season-dependent change in coliform bacteria concentrations is evident in NJ streams.

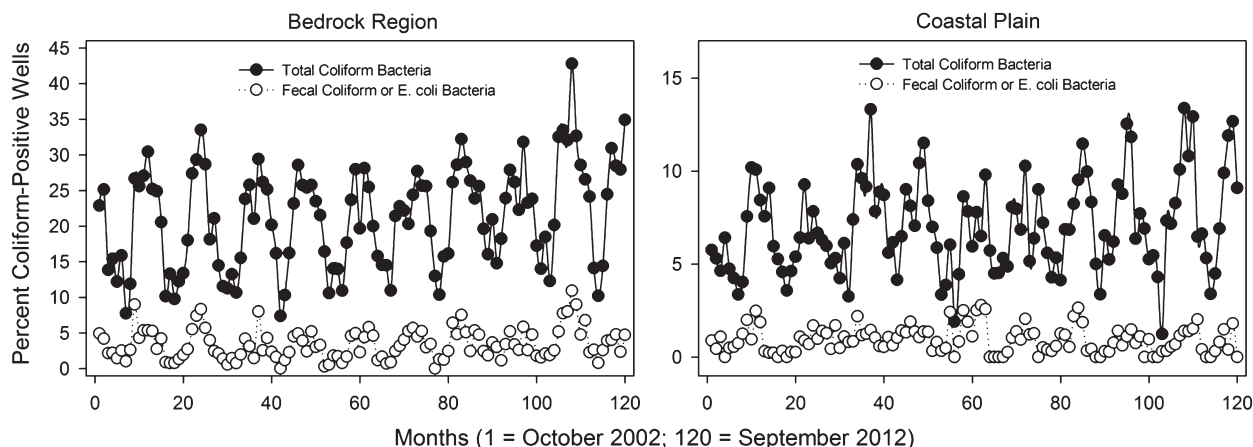


Figure 2. Percentage of NJ wells in which coliform bacteria were detected each month in the BK and CP regions.

Vegetation

The seasonal vegetation index, or “green canopy,” over NJ for the period October 2002 to December 2012, is shown in Figure 9. The observed seasonal pattern of “greenness” indicated by the vegetation index is expected for an area at the latitude of this study.

Land Use/Land Cover

GW coliform detection rates in the different land use areas and the monthly percentage of wells located on agricultural land is provided in Table S2. While there was indication that wells in agricultural areas had significantly higher detection rates than other land-use, classes the monthly pattern of the proportion of wells located in agricultural areas did not vary.

Discussion

The coliform bacteria detected in GW may be derived from a number of possible sources. GW coliform seasonality may be the result of seasonal changes in GW extraction volumes and precipitation, and/or temperature-driven changes in surface or near-surface coliform source concentrations. With regard to seasonal changes in GW extraction volumes and precipitation, seasonal changes in pathogen concentrations could mirror seasonal changes in coliform concentrations. With regard to temperature-driven changes in the surface or near-surface coliform source concentrations, seasonal changes in coliform concentrations would either not correspond to the potential presence of pathogens or do so in an inconsistent fashion.

Seasonal Changes in GW Extraction Volumes and Precipitation

Factors that could cause a seasonal change in both coliform and pathogen concentrations in GW include seasonal differences in amounts of GW extraction, and a slight increase in the amount of precipitation that occurred during the summer and fall.

Coliform sources in this category include human fecal sources, mainly from septic tanks (Yates 1985; Karathanasis et al. 2006; Donohue et al. 2015) and perhaps in a few cases, leakage from sewage collection pipes (Bradbury et al. 2013; Lee et al. 2015). However, most homes in NJ with wells are located in suburban or rural areas and hence most have on-site wastewater treatment systems (septic tanks) rather than centralized wastewater treatment facilities and their associated network of wastewater collection pipes. Animal sources include fecal wastes, via direct land inputs or stormwater infiltration. Stormwater may, on occasion, also contain human sewage (Sauer et al. 2011; Sercu et al. 2011).

Seasonal Change in the Amount of GW Extraction

Increased amounts of GW extraction during the warm weather months are mostly due to lawn and garden watering and the filling and maintenance of residential swimming pools. Based on 5 years of monthly production volumes of seven of NJ’s largest public water systems (PWS; http://datamine2.state.nj.us/DEP_OPRA/OpraMain/categories?category=Water+Allocation; 2010 to 2014 data), the amount of domestic well water used during summer (June to August) is expected to be, on average, 32% more than during winter (December to February). This percentage is the mean value of all of the PWS. During some years, at some PWS, the summer vs. winter difference was 100% or more.

Increased GW extraction volumes would result in shorter coliform and pathogen travel times from the source to the well. Shorter travel times, and shorter residence times in GW prior to extraction, would result in higher concentrations due to decreased rates of microorganism die-off due to physical, chemical, and biological factors (Gerba and Bitton 1984; John and Rose 2005). Note that die-off rates may differ between coliforms and pathogens, and among pathogens the die-off rate will be pathogen-specific.

Evidence of increased warm-weather GW extraction volumes in this study is the decrease in the average pH of well water in the BK region during warm weather

Percentage of Wells in Which Total Coliform Bacteria Were Detected

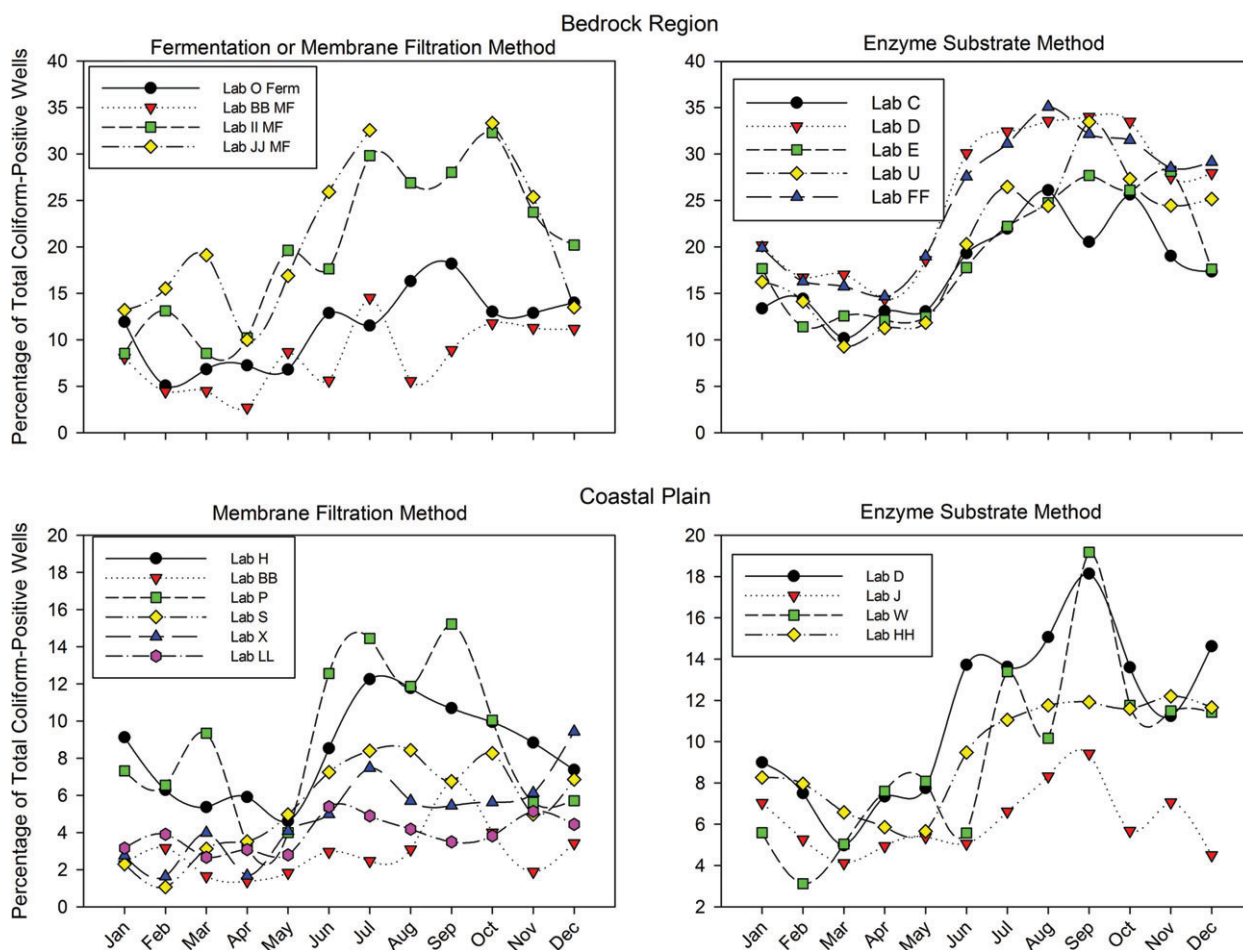


Figure 3. Monthly TC bacteria detection rates of all NJ laboratories that analyzed 100 or more samples per month. Lab JJ did not collect samples during August or September. Most of the wells analyzed by laboratories J, S, X, HH, and LL were located in the Kirkwood-Cohansey or Holly Beach aquifer portion of the CP region, where TC bacteria detection rates were lower than in wells located in the other CP aquifers (see Atherholt et al. 2013, Figure 5). Lab II collected fewer than 100 samples (range 51 to 99) in 7 of the 12 months.

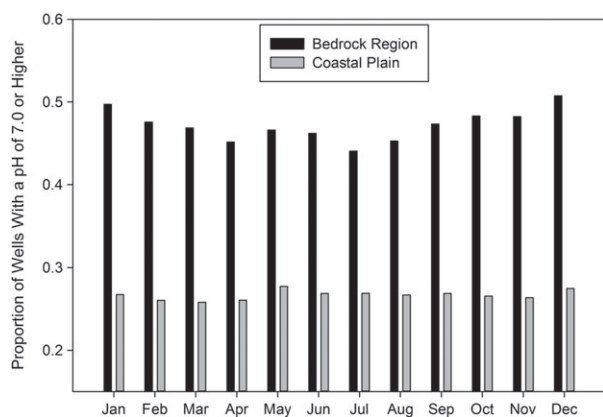


Figure 4. Proportion of NJ domestic wells with a pH of 7.0 or higher.

(Figure 4). In this region, a lower proportion of the sampled wells were basic ($\text{pH} \geq 7.0$) during warm weather than in cold weather. In the BK region, coliform detection

rates in low-pH wells are higher than those in high-pH wells (Atherholt et al. 2013), but no such seasonal effect was observed in the CP.

The warm weather decrease in the proportion of wells with a pH of 7.0 or higher in the BK region is likely the result of a change in pH of some wells resulting from a higher amount of GW extraction. That is, in the BK region, acidic precipitation (Atherholt et al. 2013) has less time to interact with neutralizing carbonate-containing rock or, in some cases, grout material, prior to extraction during the high-demand warm weather months. In the CP, most well water is already acidic, largely because of the low buffering capacity of the soils and because there is no sedimentary rock to buffer the acidic rainwater. Also, the unconsolidated strata of the CP region may fill in cracks and voids within and around grout, eliminating or reducing the ability of water residing within these cracks and voids to reach the well screen during pumping. Thus, there is no discernable seasonal difference in pH in the CP coinciding with increased water demand.

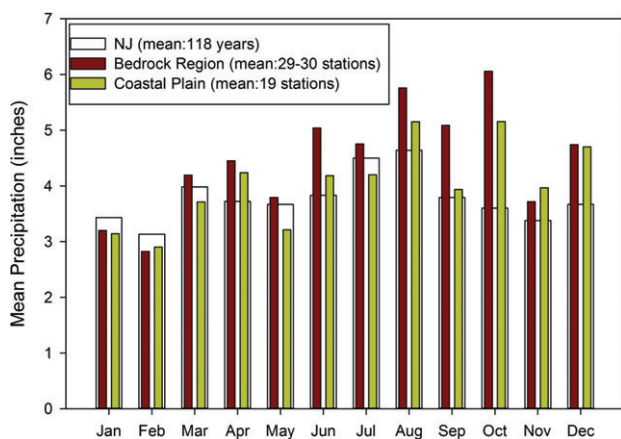


Figure 5. Monthly mean precipitation totals between October 1, 2002 and September 30, 2012 in the BK and CP regions of NJ, as well as monthly means for the years 1895 to 2013.

Precipitation

Precipitation is responsible, in most cases, for transporting microorganisms from surface or near-surface sources to GW (O'Dwyer et al. 2014). The monthly mean precipitation amounts over the 10-year monitoring period of this study, as well as monthly mean amounts over 118 years in NJ, are shown in Figure 5.

Over the past 118 years, July and August were the two months with the highest average precipitation totals in NJ. During the 10-year period of this study, the months with highest precipitation were June through October in the BK region and August and October in the CP. The 10-year August total was largely influenced by Tropical Storm Irene in 2011 (7 in [178 mm] over 24 h) along with three other multi-day storm events the same month, while the October total was largely influenced by Tropical Storm Tammy in 2005 (7 in, in the CP; 11 in [279 mm], in the BK over 72 h). Precipitation, which is ultimately responsible for the transport of coliform bacteria from the surface and near-surface sources to GW aquifers (Lamka et al. 1980; Hynds et al. 2012, 2014) was highest during the summer months, especially in the BK region. Ten-year monthly mean totals for the four seasons were BK: winter (December to February), 3.6 in (91 mm); spring (March to May), 4.1 in (104 mm); summer (June to August), 5.2 in (132 mm); and fall (September to November), 5.0 in (127 mm). Equivalent totals in the CP were: winter, 3.6 in; spring, 3.7 in (94 mm); summer, 4.5 in (114 mm); and fall, 4.4 in (112 mm). However, the seasonal differences are not large. Therefore, precipitation may have contributed to the seasonal pattern of coliform detection rates in NJ GW, but probably to a small extent compared the potential effect due to the seasonal change in GW extraction volumes. As with the seasonal difference in GW extraction rates, a seasonal difference in the amount of precipitation would be expected to exert the same effect on the infiltration rate to GW of pathogens as well as coliform bacteria.

Temperature-Driven Changes in Coliforms Derived from Surface and Near-Surface Sources

Temperature-driven changes in coliform concentrations may correspond to similar changes in a few types of pathogenic bacteria (e.g., pathogenic *E. coli*, Salmonella), but would not correspond to changes in the concentration of any viral or protozoan pathogens, or some other bacterial pathogens.

Coliform sources include the fecal-derived sources in the first category but also indigenous, or “environmentally-adapted” coliforms (Byappanahalli and Ishii 2011; Whitman et al. 2014). As septic tank disposal field laterals are only 9 to 18 inches (23 to 46 cm) below the ground surface (New Jersey Administrative Code 2012), fecal-derived coliforms from septic tank leach fields (Arnade 1999) could also undergo temperature-induced changes in concentrations. The indigenous or non-fecal sources include coliform residents of soils (Van Donsel et al. 1967; Ishii et al. 2006; Arnaud et al. 2015), sediments (Crabill et al. 1999), and coliforms contained within biofilms in surface or near-surface rock strata, and within fractures in grout and micro-annuli between the grout and well casing (Ross 2010; Kozuskanich et al. 2011; Somaratne and Hallas 2015).

Lakes, ponds, and reservoirs in the BK region may also be a temperature-influenced source of coliforms (of fecal as well as non-fecal strains) for wells located within 100 feet (30.5 m) of those waterbodies (Figure 6) (Chi-square = 31.10, $df = 4$, $p < 0.0001$ for TC and Chi-square = 23.23, $df = 4$, $p = 0.0001$ for FC/EC). Post hoc comparisons of the Chi-square tests of independence among proximity ranges to the waterbodies identified TC and FC in the BK to be far more frequently observed at wells within 100 feet than at distances further away.

Results of similar evaluations were not as explicit in the CP region, although some relationships were found (Figure 6). Chi-square tests of independence were significant for the presence of TC relative to proximity from lakes, ponds, and reservoirs as well as rivers and streams (Chi-square = 34.04, $df = 4$, $p < 0.0001$, Chi-square = 88.73, $df = 4$, $p < 0.0001$, respectively) and for FC/EC relative to rivers and streams (Chi-square = 13.10, $df = 4$, $p = 0.0108$). Post hoc tests revealed that the frequency of occurrence was different between wells within the middle proximity groups (100 to 300 feet, 300 to 500 feet) relative to those farthest away (greater than 1000 feet). Far fewer occurrences than expected were observed at the most distant wells and seemed to drive the observed proximity differences in the CP.

Temperature

Monthly median surface and ground water temperatures in NJ are shown in Figure 7. The figure shows monthly medians, but surface water temperatures exceeded 32 °C at times during July and August, and were as low as 0 °C at times in January, in some NJ streams.

Coliform bacteria are mesophiles. In laboratory experiments, coliform growth does not occur below 7.8 °C (Shaw et al. 1971; McMeekin et al. 1988). It is speculated

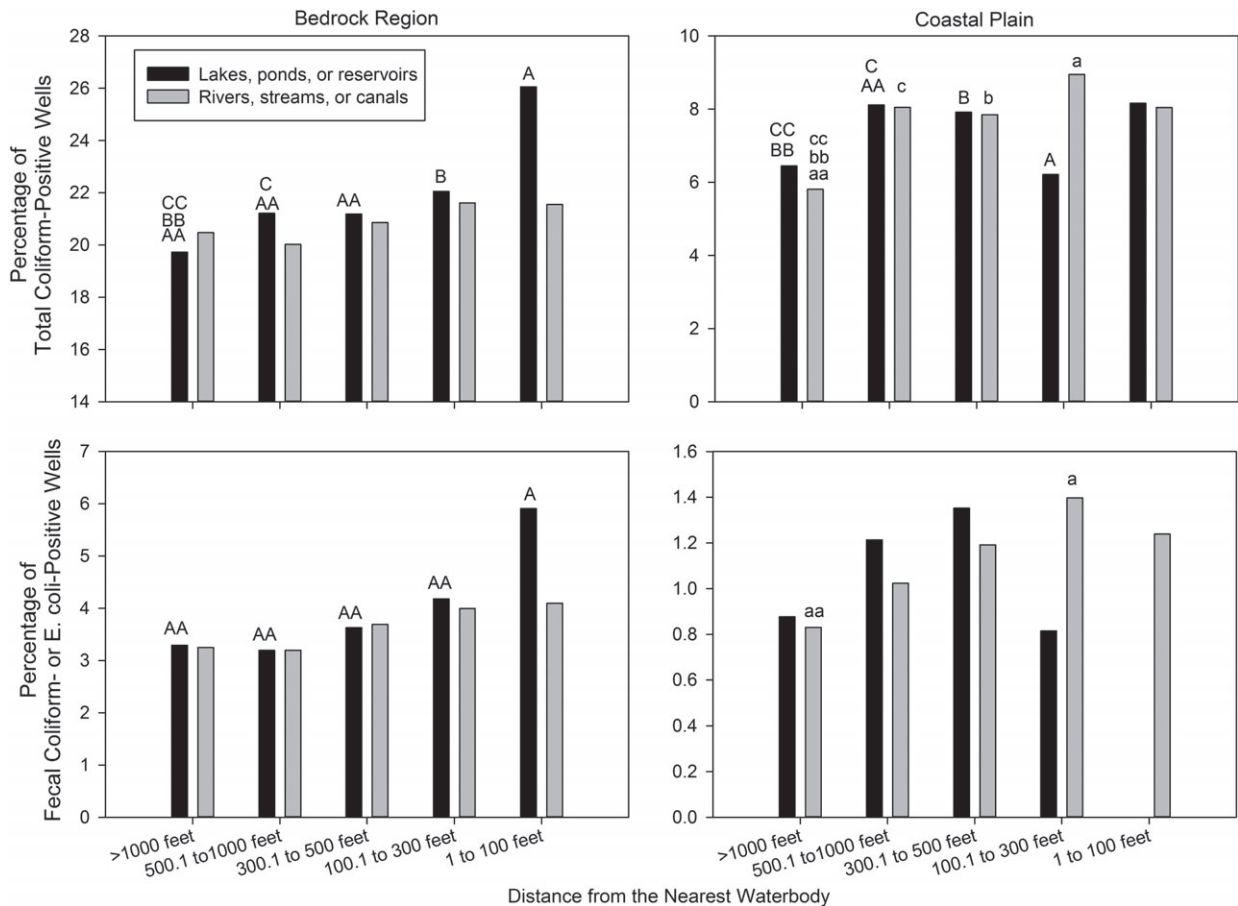


Figure 6. Percentage of NJ domestic wells in which total coliform bacteria were detected in wells located within the indicated distance from the nearest waterbody type. Repeat samples not included in this analysis. For lakes, ponds, and reservoirs, bars with “A” and “AA,” or “B” and “BB,” or “C” and “CC” differ significantly following post hoc multiple comparison tests (adjusted $p < 0.05$). For rivers, streams, or canals, bars with “a” and “aa,” etc. differ significantly.

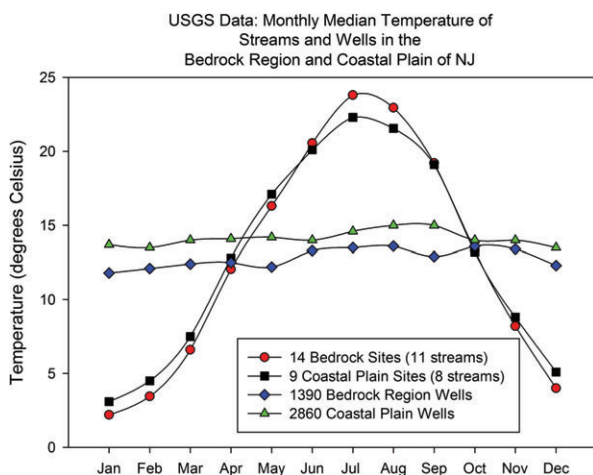


Figure 7. Monthly median temperature of monthly means of 14 monitoring stations along 11 streams in the BK region and 9 monitoring stations along 8 streams in the coastal plain region, and 1390 BK region and 2860 CP region wells in NJ. Data from the USGS.

that the comparatively low coliform concentrations in surface and near-surface coliform sources during the cold weather months is due to coliform bacteria entering the

“viable but nonculturable state” (Bjergbæk and Roslev 2005; Oliver 2016), and possibly also related to periods when frozen ground reduces GW infiltration. Bacteria in the viable but nonculturable state are metabolically active (viable) but not able to be cultured in the laboratory. Above the minimum growth temperature, coliform growth rates are very low below about 15 °C (Heitzer et al. 1991). At higher temperatures, coliform reproduction rates can be quite high under favorable environmental conditions (Brock 1970; Bronikowski et al. 2001).

Therefore, in the presence of sufficient nutrients, such as found in soils and sediments, coliforms subject to warming temperatures in the spring can multiply and increase their concentrations when their growth rate exceeds their die-off rate (due to various environmental factors). As temperature decreases in the fall, little or no growth occurs and coliform concentrations decrease as die-off rates becoming dominant (Gallagher et al. 2012) and the bacteria entering the viable but nonculturable state. It follows then that higher concentrations of coliforms are expected in the surface and near-surface environment during warm as opposed to cold months.

We do not believe that indigenous, or environmentally adapted, coliforms exist as permanent residents in GW

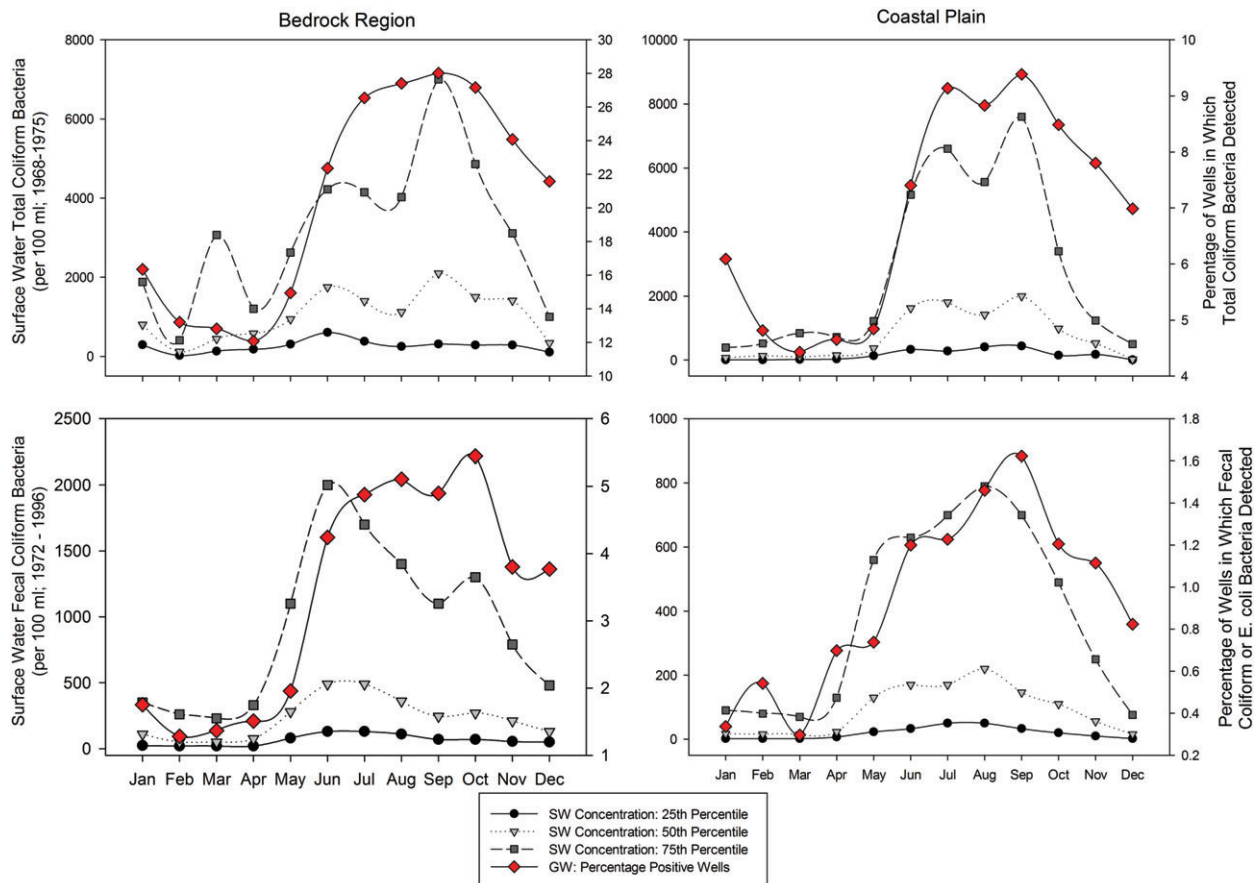


Figure 8. Coliform bacteria concentrations in NJ streams and rivers (USGS data) and percentage of domestic wells in which coliform bacteria were detected.

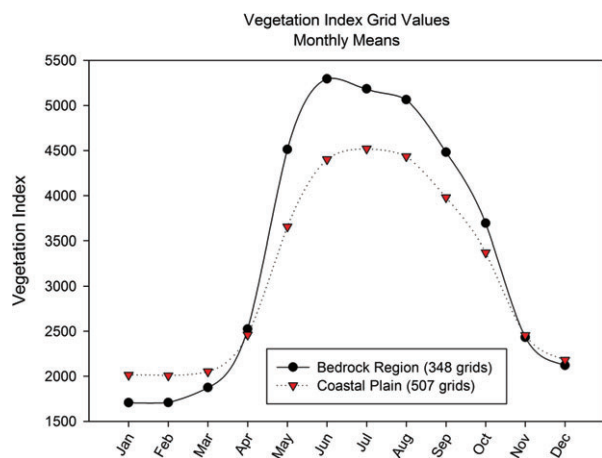


Figure 9. Monthly mean vegetation index values in the BK and CP regions of NJ.

(Personné et al. 2004). However, even if they exist, because monthly median temperatures in NJ GW do not vary greatly (Figure 7; BK region: 11.8 to 13.6 °C; CP region, 13.5 to 15.0 °C), little, if any, growth would occur and hence they would not contribute to the observed seasonality of coliform detection rates. Because seasonality in detection rates is observed, the GW coliforms must be derived from locations (surfaces and

near-surfaces) where temperature changes permit coliform concentration changes. Because the existence of GW depends on precipitation and its subsequent infiltration, the slight increase in GW temperatures during the warm weather months (Figure 7) likely reflects the influence of surface and near-surface temperatures, further supporting the influence of surface and near-surface coliform sources on observed seasonal GW concentrations (and hence detection rates).

Surface Water Coliform Bacteria

Monthly river and stream coliform concentrations and GW detection rates are shown in Figure 8. There is a clear, temperature-dependent change in coliform concentrations in NJ surface waters. The data also show that the upper 50% of the coliform concentration range is far higher during the warm weather months. As confirmation of the statewide seasonal pattern shown by the USGS surface water data, data generated by the Brick Township Municipal Utilities Authority showed the same monthly pattern of TC and FC bacteria, as well as *E. coli* concentrations, in their 70 square miles (181 square kilometers) Metedeconk River watershed (www.waterqualitydata.us/; data not shown).

Seasonal differences in surface water and stormwater coliform concentrations have been observed by many

investigators (e.g., Hunter and McDonald 1991; Hathaway et al. 2010; Duris et al. 2013; Meals et al. 2013). Such seasonality is undoubtedly a universal pattern for mesophilic organisms in all waterbodies in temperate climates (Giovannoni and Vergin 2012; Ran et al. 2013; Xia et al. 2015).

Although portions of some rivers and streams could be sources of GW bacteria, the data in Figure 6 argue against rivers and streams as a discernable source in this regional-scale study. Therefore, the stream and river bacteria concentration data in Figure 8 are provided not as a source of GW bacteria but as an indicator of similar seasonal changes in coliform concentrations that undoubtedly occur in the other potential surface and near-surface sources noted above.

Vegetation as an Additional Seasonal Coliform Source?

Other than nutrients present in soils, sediments, and decaying biological material, an additional potential seasonal nutrient source of coliforms is vegetation. The seasonal vegetation index, or “green canopy,” over NJ is shown in Figure 9. The figure is presented to provide evidence of an additional potential temperature-related coliform source.

Although the similar seasonal patterns of surface water coliform concentrations (Figure 8) and vegetation may be coincidental, a number of investigators have demonstrated associations of coliforms with algae (Byappanahalli et al. 2003; Ksoll et al. 2007) and plants (Rivera et al. 1988; Méric et al. 2013). Additionally, eukaryotic factors have been shown to induce bacterial growth (Nichols et al. 2008; Shah et al. 2008). Rainfall dislodges the bacteria from plant surfaces (Walker and Patel 1964) directly or indirectly (e.g., aerosol dissemination, stormwater runoff) to the soil, followed by GW infiltration. Rainfall may also promote growth of some bacteria on leaf surfaces (Hirano et al. 1996).

Therefore, in addition to the effect of temperature on coliform concentrations in previously enumerated surface and near-surface sources, temperature may also influence the seasonal cycle of coliform concentrations through its association with vegetation cover.

Factors Which Influence PWTa Coliform Detection Rates but Do Not Exhibit a Seasonal Pattern

A number of previously analyzed factors are known to influence the coliform detection rates including the type of geologic formation and the number of times a well is sampled (Atherholt et al. 2013, 2015). It was important to see if any of these varied in a seasonal pattern as a result of sampling “bias.”

Wells located in sedimentary rock and wells located in areas with little or no overburden due to past glacial activity have a higher percentage of coliform-positive wells than wells in other types of rock or where the overburden is thick (Atherholt et al. 2013). If a higher percentage of such wells were sampled in warm weather than in cold weather, this could account, or partially

account, for the observed seasonal increase in coliform-positive wells. However, this was not the case.

Figure S3 shows that, as expected, although the data varied from month to month, neither the proportion of wells located in more-vulnerable types of rock nor those located in the most vulnerable type of glacial-impacted areas (areas with little or no overburden) exhibited a seasonal pattern which could have influenced the observed seasonal pattern of coliform-positive wells (sedimentary vs. igneous or metamorphic rock Chi-square = 11.90, $df = 11$, $p = 0.371$; carbonaceous vs. non-carbonaceous rock Chi-square = 11.70, $df = 11$, $p = 0.379$; thin till vs. thick till Chi-square = 4.37, $df = 11$, $p = 0.958$, respectively).

In the BK region, the proportion of wells sampled more than once also did not change from month to month to any significant extent (Figure S3). Although the month-to-month differences in the proportion of wells sampled more than once in the CP region was significant based on Chi-square analysis (Chi-square = 25.81, $df = 11$, $p = 0.007$), month-to-month differences were no longer appreciable after controlling for the familywise error rate using both the sequential Bonferroni and False Detection Rate post hoc tests.

Finally, although the proportion of TC-positive wells was higher in agricultural land compared to residential land (for the FC/EC-positive well populations, this difference was significant only in the CP region; Table S2A), the monthly proportion of sampled wells located in agricultural areas versus other areas did not vary in a seasonal pattern (Table S2B). Although statistically significant differences in coliform-positive well populations were found among some of the other land-use types, some of these differences were likely due to small well population numbers, especially comparisons including the barren land class (Table S2A). Thus in NJ, seasonal agricultural practices are not likely a significant contributor to the observed seasonal pattern of coliform-positive wells.

Summary

We have demonstrated a warm weather increase in coliform bacteria detection rates in domestic wells in NJ. We offer evidence suggesting that this increase may be due to one or more factors including seasonal differences in GW extraction volumes, temperature-driven changes in coliform concentrations in surface and near-surface coliform sources, and a small increase in the amount of warm weather precipitation during the 10-year study period. The increased amount of precipitation results in increased rates of infiltration, which are further increased by warm-weather water demand due to lawn and garden irrigation and swimming pool filling and maintenance.

Potential coliform sources include fecal sources from humans and other animals. Human fecal sources include primarily septic tank effluent and, perhaps in a few cases, leaks from compromised wastewater collection pipes. Potential domestic animal sources include pets and, in

a few areas, farm animals. Indigenous animal sources include warm-blooded animals such as rodents and birds.

Potential indigenous, or environmentally adapted sources include coliforms present in soils, sediments, on plants and algae, and in fractures and gaps in near-surface rock as well as well grout and casing exteriors.

To the extent that the coliforms are derived from fecal sources whose concentrations in GW are due to seasonal changes in extraction volumes or the amount of precipitation, changes in the concentration of these bacteria may indicate similar changes in concentrations of pathogenic organisms.

However, as reviewed in Post et al. (2011), of the three major types of waterborne human pathogens (bacteria, viruses, and protozoan parasites), only bacteria are capable of growth outside their respective hosts. Thus, if the seasonal change in fecal-derived coliform bacteria in GW is due to a temperature-driven change in surface or near-surface coliform concentrations, then the change will not correspond to a change in viral or protozoan pathogen concentrations (or some bacterial pathogens as well) in a consistent fashion. Indeed, due to longer survival at lower temperatures, detection rates of viruses in GW tend to be higher during winter (Yates et al. 1985; Scandura and Sobsey 1997).

Because indigenous or environmentally adapted coliform strains are not derived from fecal wastes, or have not been for some time, detection of such bacteria in GW does not indicate the presence of fecal wastes. Thus, the utility of coliform bacteria as indicators of fecal pollution is reduced by the extent to which coliforms in GW are derived from strains that are subject to temperature-driven changes in concentration. Additional research is needed to find ways to differentiate the coliform sources. The findings of this study add further weight to the accumulating evidence (Luo et al. 2011; Sadowsky and Whitman 2011) that better indicators are needed for fecal pollution in general, and human and domestic-animal fecal pollution in particular, in GW.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Precipitation monitoring site information.

Table S2. Groundwater coliform detection rates in the different land use areas (A) and the monthly percentage of wells located on agricultural land (B).

Figure S1. Precipitation monitoring stations and the percentage of total days that were monitored between September 1, 2002 and December 31, 2012.

Figure S2. USGS surface water coliform bacteria sampling stations in NJ and the number of samples collected at each station.

Figure S3. Factors that influence coliform bacteria detection rates, but do not exhibit a seasonal pattern.

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